World Journal of Gastroenterology

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DOI: 10.3748/wjg.v3.i2.69

World J Gastroenterol 1997 June 15; 3(2): 69-71
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL RESEARCH

Effect of octreotide on cell-cycle kinetics and serum carcinoembryonic antigen level in hepatic metastases of colonic adenocarcinoma

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Author contributions: All authors contributed equally to the work.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

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Received: September 30, 1996
Revised: February 15, 1997
Accepted: April 1, 1997
Published online: June 15, 1997

Abstract

AIM: To study the inhibitory effect of somatostatin analogue (octreotide) on tumor growth.

METHODS: The influence of cell-cycle kinetics on hepatic metastases of BALB/c mice colonic adenocarcinoma (CT26) with octreotide treatment *in vivo* was investigated by flow cytometry. The serum carcinoembryonic antigen (CEA) levels were also determined.

RESULTS: The results showed that the proliferative index (PI) and the S-phase fraction in hepatic tumors of mice treated with octreotide decreased markedly and that the G_0/G_1 serum CEA phase fraction increased significantly in comparison with the control ($P < 0.01$). After administration of octreotide, the serum CEA levels were also lower than those in the control group. The incidence of liver metastases in the treated group was lower than that in the control. The body weight loss in the mice was slower and survival was longer in the treated group than in the control group. Furthermore, the changes in PI and the fraction distribution of S-phase or G_0/G_1-phase in cell cycle were closely related to the serum CEA levels.

CONCLUSION: Octreotide may be useful for inhibiting the hepatic metastases of colonic carcinoma.

Key words: Colonic neoplasms; Liver neoplasms/secondary; adenocarcinoma; Cytometry; Octreotide; Carcinoembryonic antigen/analysis

Liu R, Wang YH, Tang Y, Cao GS. Effect of octreotide on cell-cycle kinetics and serum CEA level in hepatic metastases of colonic adenocarcinoma. *World J Gastroenterol* 1997; 3(2): 69-71 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i2/69.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i2.69

INTRODUCTION

Somatostatin analogues (*e.g.* octreotide, RC-160 and others) are widely used to treat neuroendocrine tumors (such as carcinoids, vasoactive intestinal peptide (VIP) omas, insulinomas and glucagonomas), acute pancreatitis and fistulae of gastrointestinal tract[1]. Recently, these drugs have also been considered to have some therapeutic effect for non-endocrine gastrointestinal tumors[2,3]. In order to study the mechanism of octreotide and its anticancer effect, the proliferative index (PI) and the fraction distribution of cell cycle were investigated by flow cytometry. The changes in the serum carcinoembryonic antigen (CEA) level, incidence of hepatic metastases, body weight, and survival time were also determined. Furthermore, the relationship between the parameters of cell-cycle kinetics and the changes of serum CEA level were analyzed.

MATERIALS AND METHODS

Peptides, tumor cell line, and instrumentation

Octreotide was provided as a gift from Sandoz Company (Basel, Switzerland). The tumor cell line CT26, from the colonic adenocarcinoma of mice, was obtained from the Immunological Department of Second Military Medical University (Shanghai, China). The type of flow cytometry (FCM) apparatus used in this study was FACStar Plus, manufactured by Becton Dickinson Company (United States). The CEA ELISA kit was produced by Sanye Company of Shanghai Second Medical University.

Animals and treatment

Thirty-two male BALB/c mice, aged 5-7 wk, were used in this study. The mice were randomized into the following four groups: Group A ($n = 10$): Liver metastases treated with octreotide for tumor measurement and FCM analysis; group B ($n = 10$): Liver metastases used as control for tumor measurement and FCM analysis; group C ($n = 6$): Liver metastases treated with octreotide for survival time; group D ($n = 6$): Liver metastases used as control for survival time.

The mice were placed under ether anesthesia, and a small incision was made in the left subcostal area. The spleen was injected with 1×10^6 cultured CT26 in 100 μL of culture medium. The mice

Table 1 Comparison of body weight and incidence of hepatic metastases

	Group A	Group B
Body weight ($\bar{x} \pm s$, g)		
0	16.74 ± 0.94	16.96 ± 0.90
1st week	16.55 ± 0.82	16.64 ± 0.80
2nd week	16.11 ± 0.79	15.68 ± 0.68[b]
3rd week	15.35 ± 0.81[b]	15.05 ± 0.87[b]
Weight decreasing rate ($\bar{x} \pm s$, %)		
1st week	1.23 ± 0.82	1.74 ± 1.40
2nd week	3.70 ± 1.12[d]	7.48 ± 2.14
3rd week	8.25 ± 2.65[c]	11.27 ± 2.79
Incidence of hepatic tumor		
Grade Ⅰ	0	0
Grade Ⅱ	70% (7/10)[e]	20% (2/10)
Grade Ⅲ	30% (3/10)[e]	80% (8/10)

[b]$P < 0.01$ *vs* before experiment; [c]$P < 0.05$, [d]$P < 0.01$, group A *vs* group B.

Table 2 Changes in proliferative index and phase fractions in tumor cell cycle ($\bar{x} \pm s$)

	Group A	Group B
PI	0.24 ± 0.06[b]	0.31 ± 0.02
S (%)	17.89 ± 5.11[b]	24.54 ± 2.58
G_0/G_1 (%)	76.41 ± 5.51[b]	69.38 ± 2.30
G_2/M (%)	6.11 ± 1.71	5.67 ± 1.76

PI: Proliferative index. [b]$P < 0.01$, group A *vs* group B.

in groups A and C were administered daily subcutaneous injection of octreotide at a dose of 50 μg/kg daily, while those in groups B and D were injected with saline solution.

Measurement and analysis of FCM

The mice were weighed once a week. The animals in groups A and B were killed after 3 wk. The incidence of hepatic metastases and the liver weight were determined. The hepatic metastases were classified into grades Ⅰ-Ⅲ according to the number of visible liver tumors: Grade Ⅰ: 0; grade Ⅱ: 1-50 liver tumors mass; grade Ⅲ: > 50 liver tumors with mass or diffuse growth. Blood samples were collected by ophthalmectomy and the serum level of CEA was measured. Tumor samples of volume approximately 0.5 cm^3 were dissected from the hepatic metastases was subjected to FCM analysis.

The mice in groups C and D were treated continuously until they died naturally.

Statistical analysis

Statistical analysis included both univariate (χ^2 and Student's *t*-test) and multivariate (logistic regression) tests.

RESULTS

Change in body weight

The changes in the body weight of groups A and B are shown in Table 1. Compared to status before initiation of treatment, the weight in group A did not change significantly even 1 and 2 wk after treatment; however, the weight in group B decreased markedly after the second week. The difference in the rate of weight loss between groups A and B was statistically significant.

Change of hepatic metastases

Administration of octreotide for 3 wk significantly lowered the incidence of hepatic metastases and inhibited the growth of CT26 (Table 1). The incidence of grade Ⅱ liver metastases in the control group decreased markedly, while that of grade Ⅲ increased significantly in comparison with the treated group ($P < 0.05$).

PI and phase fraction in tumor cell cycle

The PI and each phase fraction of tumor cell cycle, as determined by FCM, are shown in Table 2. After administration of octreotide, the PI and the S-phase fraction in hepatic tumors of mice decreased markedly, while the G_0/G_1-phase fraction increased significantly in comparison with the control group ($P < 0.01$).

Change of serum CEA and the relationship between CEA and cell cycle kinetics

After the third treatment session, the serum level of CEA was 89.60 μg/L ± 31.72 μg/L in group A and 140.50 μg/L ± 49.97 μg/L in group B ($P < 0.05$). Statistical regression analysis showed that the changes in the PI and the fraction distribution of S-phase and G_0/G_1-phase in cell cycle were closely related to the levels of serum CEA in the treated group ($r = 0.6677$, $P < 0.05$; $r = 0.7071$, $P < 0.01$; $r = -0.6703$, $P < 0.05$, respectively).

Evaluation of survival time

After 3 wk of octreotide treatment, the median survival time was 49.3 ± 13.0 d in group A and 34.2 ± 7.3 d in group B ($P < 0.05$).

DISCUSSION

Somatostatin analogue, designated as octreotide with eight amino acids, is long acting and much more potent than somatostatin in inhibiting insulin, glucagon, and growth hormone. Clinical studies have demonstrated that octreotide is an effective pharmaceutical agent in treating various endocrine tumors (*e.g.* carcinoids, insulinomas, VIPomas, *etc.*). In recent years, several investigators have described that somatostatin analogues can exert inhibitory effect in gastrointestinal "non-endocrine" tumors by means of suppressing the growth of tumors and prolonging the survival time[4,5].

In view of cell-cycle kinetics, PI and S-phase fraction always reflect the proliferative state of tumor cells. This study demonstrated that octreotide could markedly decrease the PI and the S-phase fraction of hepatic tumors and significantly increase the G_0/G_1-phase fraction in comparison with the control. Administration of octreotide significantly lowered the incidence and inhibited hepatic metastases of CT26 colonic adenocarcinoma cell line. The animal's body weight decreased slowly and the survival time of mice prolonged markedly. Therefore, octreotide can inhibit the proliferation of CT26 tumor cell line and block the G_0/G_1-phase cells of CT26 adenocarcinoma from entering the S-phase. These experimental results from the view of cellular kinetics suggest that octreotide has the antitumor effect.

The possible mechanism of the antitumor action of somatostatin is still unknown. From the available data, the following are proposed as likely mechanisms: (1) The antitumor action of somatostatin could be mediated directly by specific receptors located on the tumor cell membrane. (2) Somatostatin analogues can suppress the release of gastrointestinal hormones (*e.g.* insulin, gastrin, cholecystokinin and others), interfere with the synthesis of autocrine or paracrine growth factors by tumor cells (such as epidermal growth factor, platelet derived growth factor, transforming growth factor and others. (3) Somatostatin also exerts an inhibitory effect on DNA synthesis in tumor cells. And (4) Furthermore, somatostatin analogues can also inhibit the angiogenesis of neoplasm[6-8].

Our data suggested that octreotide can also influence serum CEA. The serum CEA levels in the group treated with octreotide decreased markedly and were positively related with PI and S-phase fraction of cell cycle and negatively related with G_0/G_1-phase fraction. CEA, an oncofetal glycoprotein, was first described in 1965. The present study shows that the CEA is not only a tumor associated cancer antigen but also a member of immunoglobulin supergene family[9]. The nucleotide sequence of the CEA gene was homologous with those of member of the immunoglobulin supergene family; therefore, it may affect cellular recognition and interaction. As a member of the group of cell adhesion molecules (CAMs), CEA may be involved in tumor metastases. The experimental study by Hostetter *et al*[10] suggested that the metastasis in the liver of nude mice was enhanced by the CEA pretreatment. The cells adhered by movement of microvilli and contact of cells. The recognition between CEA molecule and its receptor is a process depending on bivalent cation (Mg^{2+})[11]. Therefore, this study suggested that the anti-tumor mechanism of octreotide might involve cell adhesion molecules (CAMs). Unfortunately, we did not determine the changes in the levels of other CAMs.

In conclusion, our findings indicate that the somatostatin anal-

ogue, octreotide, can effectively inhibit the growth and development of hepatic metastases. Recently, octreotide has been employed in the treatment of advanced gastrointestinal cancer patients refractory to chemotherapy. Although the results of initial clinical trials of somatostatin analogues were controversial, we believe that somatostatin may be a useful agent in endocrinotherapy for gastrointestinal cancer.

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S- Editor: Filipodia **L- Editor:** Jennifer **E- Editor:** Liu WX

Published by **Baishideng Publishing Group Inc**
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ISSN 1007-9327